

Views and Reviews

Microtubule-Actomyosin Interactions in Cortical Flow and Cytokinesis

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INTRODUCTION

Cytokinesis in animal cells is simultaneously one of the most fascinating and frustrating of cellular phenomena. The fascination stems from its precision and importance, while the frustration stems from its complexity and the wealth of apparently contradictory information about the cellular and molecular mechanisms required for proper cell division [Rappaport, 1996]. Although it is generally accepted that a cortical network of actomyosin provides the force for cell fission, and that microtubules are required for the assembly and positioning of the actomyosin network, the means by which microtubules control the actomyosin cytoskeleton is poorly understood and therefore hotly debated.

Cytokinesis can be conceptually divided into three phases: cytokinetic apparatus assembly, furrow progression, and fission completion. Furrow assembly and furrow progression ensue as actomyosin becomes concentrated in the equatorial region as a result of cortical flow, the movement of cortical f-actin, myosin-2, and cell surface proteins [e.g., Wang et al., 1994] toward the site of the forming furrow. Fission completion results when the two daughter cells are completely separated, and may occur minutes to hours after the onset of furrowing. This review is concerned with the first two of these phases and, in particular, the means by which microtubules specify the assembly of the actomyosin apparatus that drives cytokinesis.

Two important assumptions underlie the discussion that follows. The first is that furrowing is primarily dependent on paired arrays of microtubules rather than any special feature of the mitotic spindle. This assumption is based on the demonstration that in embryos

[Rappaport, 1996], *Dictyostelium* [Neujahr et al., 1998], and cultured cells [Rieder et al., 1997] furrowing occurs between adjacent asters that lack an intervening spindle. Thus, neither midzone microtubules nor chromosome-associated microtubules are required for the initiation of furrowing, although midzone microtubules are apparently required for the completion of cytokinesis [e.g., Savoian et al., 1999].

The second assumption is that while cytokinesis is normally entrained to exit from M-phase, the basic microtubule-actomyosin interactions that result in furrow assembly and progression are operative throughout much of the cell cycle. This assumption is based on both the demonstration that cytokinesis can be extended well into interphase by physical [Rappaport, 1996] or pharmacological [Martineau et al., 1995] manipulations, and that cytokinesis occurs in echinoderm embryos locked into M-phase by injection of nondegradable cyclin when the mitotic apparatus is displaced toward the cortex [Shuster and Burgess, 1999]. Thus, in our view, exit from M-phase is important mainly insofar as it impacts the spatial relationship of microtubules with the cortex. If these assumptions are accepted, then rules established for microtubule-actomyosin interactions in interphase cells

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are directly relevant to our understanding of the mechanisms that control cytokinesis.

ASTRAL INHIBITION VS. ASTRAL STIMULATION

One of the central debates in experimental and theoretical studies of cytokinesis is whether microtubules stimulate or inhibit actomyosin-based contraction. The “astral inhibition” model presupposes that microtubules inhibit actomyosin-based contraction, and that the cytokinetic apparatus, therefore, accumulates between opposing asters, where microtubules are least numerous. Support for this model comes from theoretical [White and Borisy, 1983] and empirical [Asnes and Schroeder, 1979] demonstrations that microtubules are least abundant at the site of furrow formation [but see also Danilchik et al., 1998 and citations in Devore et al., 1989]. In its best-known formulation, the astral inhibition model assumed that the negative influence of microtubules is primarily imposed on the poles [i.e., the areas most distal to the forming furrow; White and Borisy, 1983].

The “astral stimulation” model, on the other hand, presupposes that microtubules stimulate actomyosin-based contraction, and that the cytokinetic apparatus therefore accumulates between opposing asters, where microtubule ends are proposed to be most numerous [Harris and Gewalt, 1989; Devore et al., 1989]. This model is supported by the finding that experimental increases in the distance between the poles and the spindle asters or imposition of physical barriers between the poles and the asters does not prevent furrowing [Harris and Gewalt, 1989; Devore et al., 1989; Rappaport, 1996]. Based on mathematical modeling, it was proposed that microtubules stimulate actomyosin-based contraction in a manner that can be best explained by a sigmoidal relationship [Harris and Gewalt, 1989]. That is, the ability of paired asters to trigger furrowing occurs over a very narrow window of distance between the asters and the cortex. When the asters are at that threshold distance, or are closer, the stimulatory effect is at 100%. When asters are only slightly beyond that threshold distance, the stimulatory effect is at 0% (Fig. 1; see also fig. 5 in Harris and Gewalt). Such sigmoidal relationships are hallmarks of positive feedback loops, wherein an initially small signal is rapidly amplified by autocatalysis.

MICROTUBULES SUPPRESS ACTOMYOSIN-BASED CONTRACTION

The demonstration that manipulating the relationship between the poles and the spindle asters has no effect on furrowing is, in our eyes, compelling evidence that poles per se are not necessarily important for furrowing activity. However, based on direct analyses of the effects of microtubules on actomyosin-based contraction in a

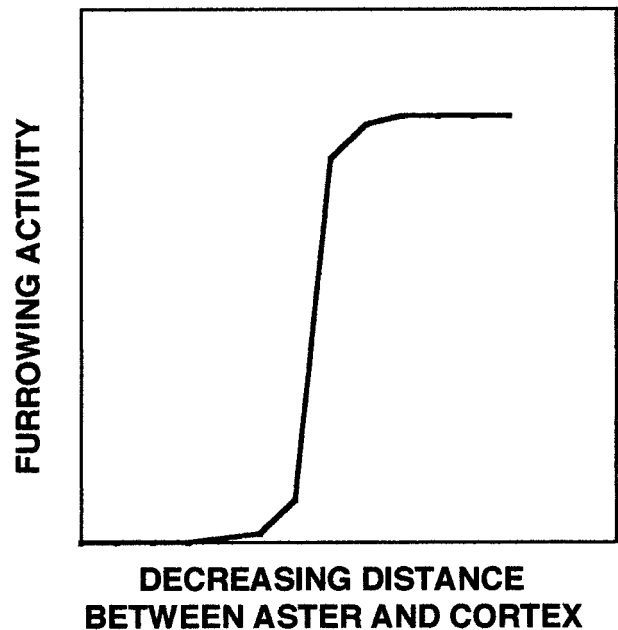


Fig. 1. Schematic plot showing sigmoidal relationship between the distance of the asters from the cortex and the ability of the asters to trigger furrowing. The effect is negligible until the asters are within a certain distance, and at that distance or closer, the effect is maximal. Modified from Harris and Gewalt, 1989.

variety of different cell types and situations, we suggest that microtubule-dependent inhibition of actomyosin-based contraction is still likely to account for the accumulation of actomyosin in the nascent cleavage furrow.

First, microtubule depolymerization stimulates actomyosin-based contraction in every cultured cell studied to date [e.g., Lyass et al., 1988; Danowski, 1989; Kolodney and Elson, 1995]. Conversely, stabilization of microtubules inhibits actomyosin-based contraction in cultured cells [Danowski, 1989]. Second, the rate of actomyosin-based cortical flow is inversely proportional to the amount of polymerized tubulin [Canman and Bement, 1997] while microtubule depolymerization accelerates contraction of cortical f-actin in *Xenopus* oocytes (Benink et al., unpublished data). Third, microtubule depolymerization accelerates both furrow progression in cells undergoing cytokinesis [Hamilton and Snyder, 1983] and closure of wound-induced actomyosin purse strings in *Xenopus* oocytes [Bement et al., 1999]. Fourth, cortical flow moves away from microtubule organizing centers in both *C. elegans* embryos [Hird and White, 1993], and *Xenopus* oocytes (Benink et al., unpublished data).

ACTION AT A DISTANCE: MICROTUBULE-BASED SUPPRESSION OF CONTRACTILITY VIA RHO AND RAC

The molecular underpinnings of microtubule-based suppression of actomyosin contraction are just now being

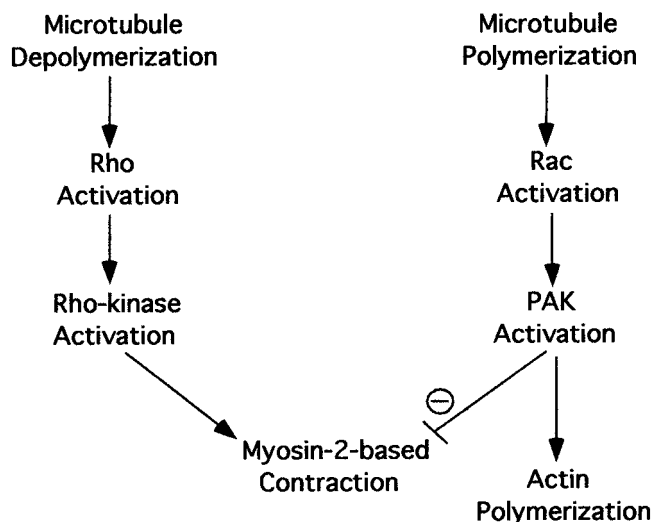


Fig. 2. Schematic diagram outlining the effects of microtubule depolymerization and polymerization on rho and rac, respectively. *Arrows* indicate positive interaction; *minus symbol* indicates negative interaction.

characterized. Microtubule depolymerization results in activation of the small GTPase, rho [Ren et al., 1999], which results in stimulation of myosin-2-dependent contractility. Specifically, rho activates rho-dependent protein kinase, which suppresses dephosphorylation of the myosin-2 regulatory light chain (RLC) by the RLC phosphatase [Kimura et al., 1996]. Thus, microtubule depolymerization can stimulate actomyosin-based contraction via rho activation. Importantly, a requirement for rho in the regulation of cytokinesis has been directly demonstrated in several systems [e.g., Mabuchi et al., 1993; Dreschel et al., 1997; O'Connell et al., 1999].

Microtubule polymerization also impacts the actin cytoskeleton, but in a manner that is opposed to contraction. That is, microtubule polymerization stimulates rac, another small GTPase [Waterman-Storer et al., 1999]. Rac activation results in actin polymerization [Machesky and Hall, 1997] and RLC dephosphorylation via inhibitory phosphorylation of myosin light chain kinase by PAK, a kinase activated by rac-GTP [Sanders et al., 1999]. Thus, areas of low microtubule density should be relatively contractile, while areas of high microtubule density should be relatively noncontractile (Figs. 2, 3).

Exactly how microtubules influence rho and rac is unknown. However, microtubules can trigger plasma membrane ruffling (which is rac and actin filament polymerization-dependent) while at a distance from sites of ruffling [Waterman-Storer et al., 1999], implying that mechanisms acting by way of rac and rho do not require direct coupling of the microtubule and actomyosin cytoskeletons. This presumption is consistent with several studies that have shown an influence of microtubules on

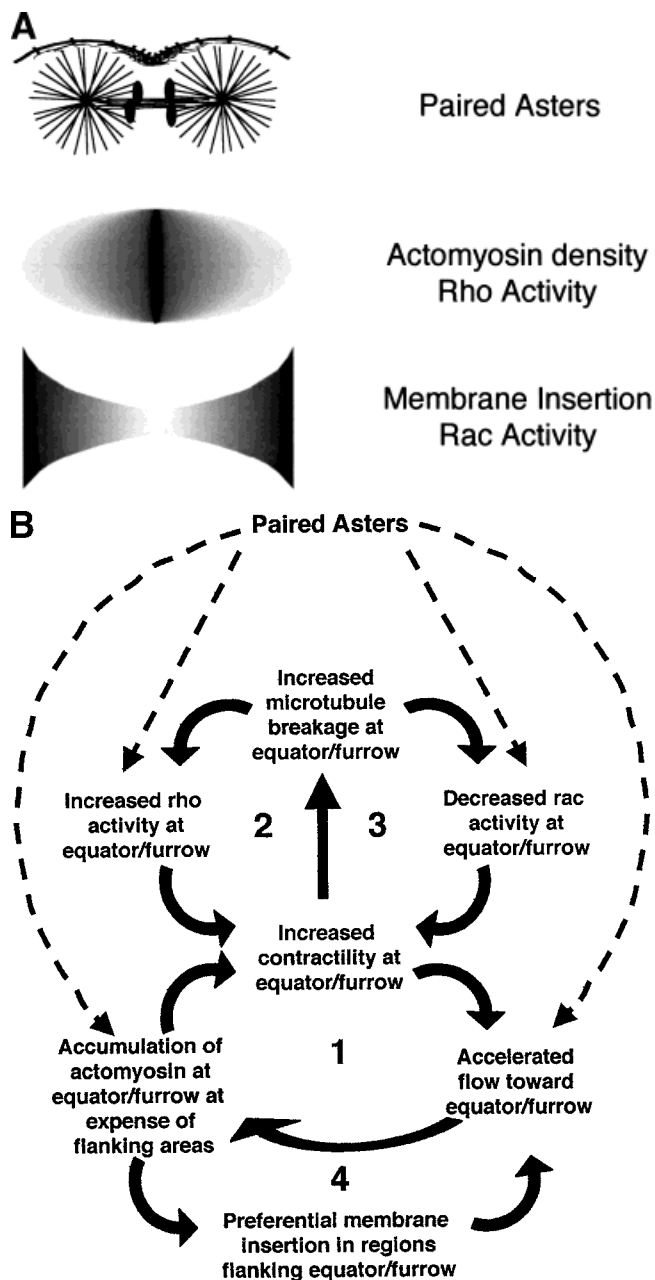


Fig. 3. **A:** Schematic diagram depicting the relationship of the asters, the forming cytokinetic apparatus, and predicted gradients of actomyosin density, rho activity, rac activity, and membrane insertion. Peak activity is represented by dark, wide lines; minimal activity is represented by light, narrow lines. **B:** Schematic diagram showing potential positive feed back loops in the proposed model for cytokinesis. Loops connected by *solid arrows* represent those that do not require continuous input from the asters. The paired astral array feeds into the loops at several points, indicated by *dashed arrows*.

the f-actin cytoskeleton in the apparent absence of contact between the two [e.g., Danowski, 1989; Mikhailov and Gundersen, 1998] as well as models for microtubule-dependent regulation of cell locomotion via rac and rho [Waterman-Storer and Salmon, 1999].

ACTION VIA DIRECT COUPLING: MICROTUBULE-F-ACTIN ASSOCIATION

In addition to suppressing contraction via regulators of the actomyosin cytoskeleton, direct coupling of the microtubule and f-actin cytoskeletons may also control cortical flow and cytokinesis. A number of proteins and protein complexes with the potential to link the two systems have been identified [e.g., Goode et al., 1999], and association of the two systems has been documented in cell free *Xenopus* egg extracts [Sider et al., 1999]. Physical linkage between the two systems could passively lead to suppression of cortical flow, since binding of f-actin to the relatively stiff microtubules physically hinders actomyosin-based contraction. Direct linkage could also actively influence flow if the combined interaction of microtubules, f-actin, and their respective motor molecules altered patterns of f-actin distribution. In a preliminary report, we showed that f-actin is actively translocated away from microtubule organizing centers in *Xenopus* egg extracts [Waterman-Storer et al., 1998]. This translocation is dynein-dependent and would be expected to promote accumulation of f-actin around the periphery of asters *in vivo*. Evidence also suggests that direct linkage between the two systems results in microtubule bending and breaking [Waterman-Storer and Salmon, 1999; Sider et al., 1999], an occurrence that could further contribute to the rapid assembly of the contractile apparatus (see below).

FURROW ASSEMBLY

The differential distribution of microtubules in anaphase and the action of rac and rho provide a simple mechanism not only for accumulation of actomyosin at the equator, but also to ensure that a continual supply of f-actin is available for recruitment into the furrow. Areas flanking the presumptive furrow are microtubule rich [Asnes and Schroeder, 1979], which would be predicted to lead to low rho activity and high rac activity. Thus, areas flanking the furrow are expected to exhibit relatively high levels of f-actin polymerization. Further, because rac inhibits RLC phosphorylation, such areas are expected to be relatively contraction-incompetent. In the microtubule-poor region between the asters, however, rho activity is expected to be high, resulting in high contractility, since rho stimulates RLC phosphorylation (Fig. 3A). This provides the initial bias required for cytokinetic apparatus assembly, such that f-actin newly polymerized in the microtubule-rich regions flanking the furrow is recruited via contraction into the microtubule-poor region at the equator. Further, the mutual antagonism of rac and rho would tend to amplify any initial difference created as a result of differential microtubule distribution and polymerization.

POSITIVE FEEDBACK LOOPS

The sharp, sigmoidal influence of microtubules on furrowing activity revealed by the theoretical studies referred to above (Fig. 1) [Harris and Gewalt, 1989] is characteristic of one or more positive feedback loops, wherein an initial, small asymmetry is amplified by autocatalysis. As described below, positive feedback loops are expected to result from cortical flow, actomyosin-microtubule interactions, and differential membrane insertion. One positive feedback loop is driven by the process of cortical flow itself [Oegema and Mitchison, 1997]. That is, an initial accumulation of actomyosin is expected to lead to the recruitment of more actomyosin to the site of the initial recruitment via cortical contraction, assuming that the actomyosin network is physically linked throughout the cortex (Fig. 3). Further, the removal of actomyosin from cortical regions outside the furrow will naturally lead to decreased tension and contractility in such regions, thereby accelerating contraction in the furrow region and, hence, further actomyosin recruitment to the equator (Fig. 3B, loop 1). This loop would be further accelerated as a result of f-actin clearing from microtubule organizing centers.

A second positive feedback loop is driven by direct linkage of the f-actin and microtubule cytoskeletons. Linkage to microtubules slows contraction and flow in areas of high microtubule density, resulting in relatively higher contraction and flow rates in areas of low microtubule density and, hence, an increase in actomyosin density in such regions. Microtubule breakage in regions of high actomyosin density and contraction reduces the relative abundance of microtubules in these regions, further speeding contraction. Breakage in the furrow region would also promote microtubule depolymerization and, hence, further rho activation and greater contractility (Fig. 3B, loop 2). A third positive feedback loop would presumably result from the fact that microtubule breakage should also lower rac activity, thereby further increasing the contractility in this region (Fig. 3B, loop 3).

A fourth positive feedback loop would be expected to result from differential insertion of new membrane into the plasma membrane. That is, several lines of evidence indicate that plasma membrane turnover both accompanies and is important for cytokinesis [e.g., Danilchik et al., 1998], and perturbation of syntaxin expression or function inhibits cytokinesis in both *C. elegans* [Jantsch-Plunger and Glotzer, 1999] and sea urchin embryos [Conner and Wessel, 1999]. The notion is that new membrane insertion facilitates furrow progression, presumably by reducing cortical tension in areas flanking the furrow. Work in other systems has shown that exocytosis is inhibited when the cortical f-actin network is experimentally strengthened and, conversely, stimulated by experi-

mental decreases in cortical f-actin [e.g., Muallem et al., 1995]. Thus, areas outside the furrow would be inherently subject to greater membrane insertion than the furrow itself. As actomyosin accumulates in the furrow, exocytosis would decrease in the furrow area, and increase in areas flanking the furrow. This would facilitate contraction and accumulation of actomyosin within the furrow, and further reduce exocytosis (Fig. 3B, loop 4).

This model has several strengths. First, it is based on mechanisms of microtubule-actomyosin interactions that have clear experimental support from studies using a variety of cell types. Second, the presence of multiple, interlocking positive feedback loops accounts for the sharply sigmoidal nature inferred for the ability of asters to trigger furrowing in a theoretical study of cytokinesis [Harris and Gewalt, 1989]. Further, while that study was based on the assumption that microtubules stimulate actomyosin-dependent contraction, it was noted that an inhibitory interaction could also account for the effects of microtubules on furrowing, as long as the influence was sigmoidal in nature [Harris and Gewalt, 1989, pp. 2219 and 2222]. Third, positive feedback between the microtubule and actomyosin cytoskeletons explains why manipulations of the f-actin cytoskeleton can perturb the normal patterns of microtubule reorganization that accompany cytokinesis [Giansanti et al., 1998; but see also Savoian et al., 1999] and explains the observation that induction of ectopic actomyosin purse strings by wounding results in microtubule reorganization [Bement et al., 1999]. Fourth, this model makes several testable predictions: (1) Rac activity should be high in areas flanking the furrow while rho activity should be high at the equator. (2) Disruption of the normal distribution of microtubules should alter the spatial patterns of rac and rho activity. (3) Membrane turnover should be highest in regions flanking the furrow and lowest in the furrow itself. (4) Experimental disruption of dynein activity should slow or inhibit cytokinesis.

We propose that the assembly of the contractile apparatus and the subsequent progression of the cytokinetic furrow can be explained by mechanisms of microtubule-actomyosin interaction found in cells that are not themselves undergoing cytokinesis. Paradoxically, many of the effects of microtubules on actomyosin-based contraction are fundamentally inhibitory. However, the region of low microtubule density flanked by regions of high microtubule density created by paired asters brings to bear several integrated positive feedback loops that convert this negative stimulus into an autocatalytic accumulation of contractile material between the asters.

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